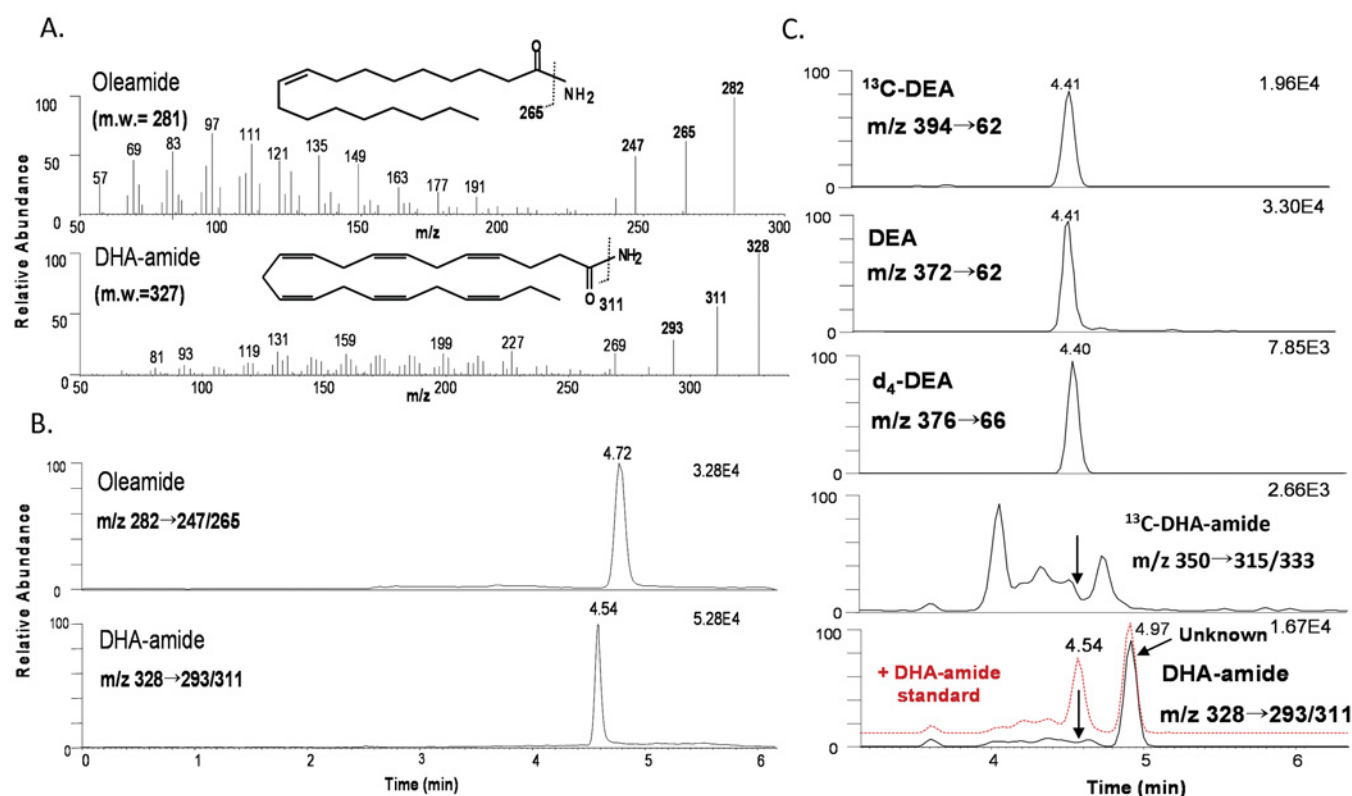


# SUPPLEMENTARY ONLINE DATA

## N-Docosahexaenoylethanolamide promotes development of hippocampal neurons

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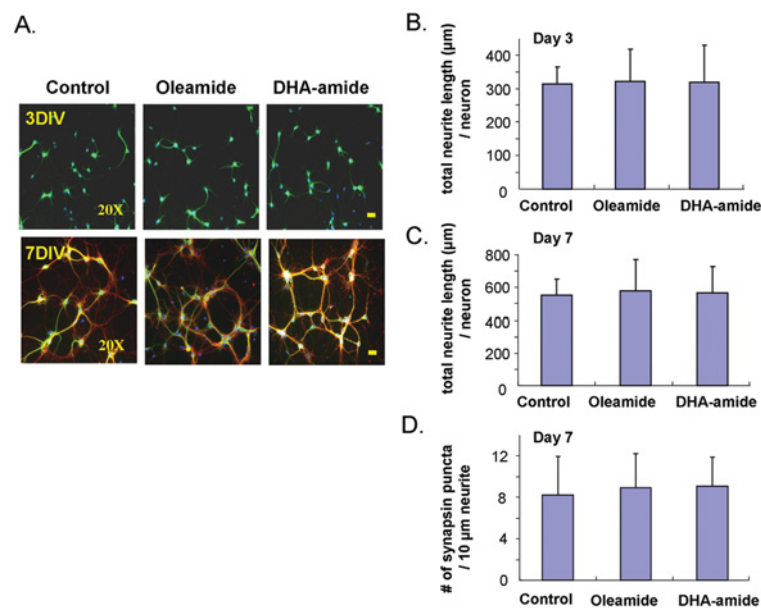
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**Figure S1** Undetectable transformation of DHA to DHA-amide in E18 hippocampal neuronal cultures

(A) MS/MS spectra obtained from 50 fmol each of standard oleamide and DHA-amide, showing characteristic fragments. m.w., molecular mass. (B) MRM mass ion chromatograms for standards using specific transitions from  $[M+H]^+$  to these characteristic fragments. (C) MRM ion chromatograms for the detection of DHA-amide in E18 hippocampal neuronal cultures after supplementing with 1  $\mu$ M each of DHA and [<sup>13</sup>C<sub>22</sub>]DHA for 3 days. Although the MRM chromatograms clearly indicated DEA production, DHA-amide formation was not detected. The arrows represent the expected retention time of DHA-amide (4.54 min), which is also indicated by the appearance of the chromatographic peak for DHA-amide after doping with DHA-amide standard.

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**Figure S2 Effect of DHA-amide on hippocampal neuronal development**

(A) Representative photomicrographs shown for E18 mouse hippocampal neuronal cells cultured with or without oleamide (1  $\mu\text{M}$ ) or DHA-amide (1  $\mu\text{M}$ ) for 3 or 7 DIV (days in *in vitro* culture). MAP2 immunofluorescence is shown in green, synapsin-1 in red and DAPI in blue. Scale bars, 30  $\mu\text{m}$ . (B–D) Effect of oleamide and DHA-amide on neurite growth and synaptogenesis. Neither neurite growth at day 3 (B) or day 7 (C) nor synaptogenesis evaluated by the number of synapsin puncta per 10  $\mu\text{m}$  of neurite at day 7 was significantly affected by these agents.